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# Both novelty and conspicuousness influence selection by mammalian predators on the colour pattern of Plethodon cinereus (Urodela: Plethodontidae)

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# Title: Both novelty and conspicuousness influence selection on *Plethodon cinereus* (Urodela: Plethodontidae) color pattern by mammal predators

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## Abstract

Predators influence the evolution of color pattern in prey species, yet how these selective forces might differ among predators is rarely considered. In particular, prey color patterns that indicate unpalatability to some predator species may not carry the same signal for other predators. We test several hypotheses of selection on patterning between mammal predators and the polymorphic salamander *Plethodon cinereus*, which, under an avian visual system appears as a mimic of the toxic newt *Notophthalmus viridescens*. We fit each hypothesis against field observations of mammalian attacks on salamander clay replicas. We then develop a novel analytical procedure that enables the combination of multiple non-exclusive models in a likelihood framework. We find that mammals do not follow any single hypothesis proposed, including the hypothesis of mimicry. Instead, mammals in this system use visual cues while foraging to avoid unfamiliar, novel prey and attack conspicuous prey. We propose that mammals may help to maintain color pattern polymorphism within populations of *P. cinereus* by avoiding novel, unfamiliar color morphs. Additionally, selective pressures from multiple predators and variation in predator communities among sites may contribute to the maintenance of color polymorphism within and among localities in this salamander species.


Keywords: *Plethodon* – non-exclusive hypotheses – mimicry – color polymorphism – clay replicas

Predation exerts strong selective pressures on prey phenotype, often acting as a creative force driving the evolution of new phenotypic traits that deter predators (Endler, 1988). For example, defensive structures present in prey populations that are sympatric with predators are frequently lost in predator-poor environments (Bell, Orti, Walker & Koenings, 1993; Pafilis *et al*., 2009; Reimchen, 2000), and experimental evidence has shown that the presence of predators exerts strong selective pressures on life history and other phenotypic traits (Reznick, Bryga & Endler, 1990; Reznick *et al.,* 1997). High rates of predation can drive prey species to mature at smaller sizes (Reznick and Endler, 1982) or to evolve extremely high toxicity (Brodie Jr., Ridenhour & Brodie III, 2002). However, while such studies inform on how predation can act as a constructive force of selection that drives the evolution of new phenotypes, less attention has been given to how predation can maintain standing phenotypic diversity within prey species (Bond, 2007).

A common phenotypic trait to evolve in response to predation is patterning, which can directly influence predator detection and identification of potential prey (Endler, 1986). In many species, selection from predators has resulted in color pattern polymorphism, where multiple distinct morphs exist for a single prey species (reviewed in Bond, 2007). The evolution of such polymorphism is strongly directed by the visual capabilities of potential predators, which can vary greatly among species (Ruxton, Sherratt & Speed, 2004). Once detected, the behavioral response of a predator to potential prey can fit several different hypotheses, which may or may not maintain color pattern polymorphisms. First, predators may avoid prey color pattern morphs that resemble an

unpalatable species, thus driving the evolution of Batesian mimics that closely resemble their unpalatable models (the Mimicry Hypothesis, Figure 1A; Bates, 1862). Alternatively, foraging predators may detect and target prey whose patterning has greater contrast with the background environment (i.e. more conspicuous), thereby driving the evolution of cryptic morphs difficult for predators to initially detect (the Conspicuousness Hypothesis, Figure 1B; Endler, 1978). Predators may also recognize, and then attack, those prey that are most common in the environment, resulting in frequency-dependent selection against common morphs (the Apostatic Selection Hypothesis, Figure 1C; Allen, 1988). On the other hand, predators may avoid unfamiliar prey (the Novel Avoidance Hypothesis; also called 'dietary conservatism', Figure 1D; Marples *et al.,* 2007), or predators may not use patterning in any way during foraging, perhaps instead utilizing other senses, such as smell (the Non-Visual Hypothesis; Endler, 1986; Hughes *et al.,* 2010; Ruxton, 2009). Importantly, these hypotheses are not necessarily exclusive. Predators may incorporate several of the above strategies into a single composite behavior when responding to visual cues in potential prey (the Combined Hypothesis; Endler, 1986). These hypotheses can be difficult to observe directly, and are best tested through the observation of predation events themselves (e.g. Kikuchi & Pfennig, 2010).

In salamanders, predation is a major source of mortality, and a diversity of patterning has consequently evolved in response to predators (Petranka, 1998). In some taxa, this includes color pattern polymorphism, or multiple color morphs, within species. In the salamander *Plethodon cinereus*, three distinct color morphs are typically encountered, including the solid red-orange 'erythristic' morph, the solid black 'unstriped' morph, and

the 'striped' morph that possesses a single red dorsal stripe on a dark background (Lotter & Scott, 1977; Figure 2A-C). Birds are hypothesized to associate erythristic *P. cinereus*, which are palatable (Tilley, Lundrigan & Brower, 1982), with similarly colored juveniles of the highly toxic newt *Notophthalmus viridescens*, making erythristic *P. cinereus* Batesian mimics of *N. viridescens* (Lotter & Scott, 1977; Brodie & Brodie, 1980; Tilley *et al.,* 1982). A recent study found that bird predators with tetrachromatic vision are able to discriminate non-mimetic *P. cinereus* from *N. viridescens*, but not mimetic *P. cinereus* from *N. viridescens* on the basis of coloration (Kraemer & Adams, 2014). Birds are thus capable of imposing selection on *P. cinereus* patterning consistent with Batesian mimicry. In contrast, this study found that visual models for diurnal, dichromatic mammals are unable to discriminate salamanders on the basis of coloration, suggesting that visual constraints might prevent diurnal mammal predators from selecting for mimicry in this system. However, these visual models indicated that mammals are able to discriminate salamanders on the basis of brightness. Mammalian predators may thus use visual cues distinct from coloration when hunting, thereby selecting for patterning in a manner different from birds.

In the present study, we test five hypotheses of selection on *P. cinereus* color pattern by mammal predators, specifically the Mimicry, Conspicuousness, Apostatic Selection, Novel Avoidance, and Non-Visual hypotheses as described above. We test the fit of each hypothesis against field observations of mammalian predation on clay replicas of each *P. cinereus* color morph. We then use a novel analytical procedure that, given a set of non-exclusive explanatory models, enables us to identify a 'combined' model that

incorporates aspects of multiple predation hypotheses within a single likelihood framework.

## Materials and Methods

### *Study System*

The salamander species *Plethodon cinereus* is the most common vertebrate species in New England forests (Burton & Likens, 1962), and many populations have two of the three typical *P. cinereus* color morphs (Lotter & Scott, 1977). While avian predators are thought to drive the evolution of color mimicry in this system (Brodie & Brodie, 1980; Tilley *et al.,* 1982; Kraemer & Adams, 2014), the impacts of additional predators, such as mammals and snakes, are less well understood. In North America, mammals are important salamander predators (Petranka, 1998). However, mammalian attacks on salamanders are harder to observe and are less well documented as compared to attacks from snakes and birds (e.g. Lotter & Scott, 1977; Arnold, 1982; Fenster & Fenster, 1996; Adams, 1999). Nonetheless, prior work has shown that common mammalian predators on salamanders are opossums, raccoons, skunks, opportunistic rodents, and other small carnivorous mammals (Stebbins, 1954; Huheey & Stupka, 1967; Brodie Jr., Nowak, Harvey, 1979; Beachy, 1991; Dodd, 1991; Petranka, 1998). These typically dichromatic mammalian species have visual capabilities far different from bird predators, particularly through a narrower range of visual sensitivities (Chen & Goldsmith, 1986; Jacobs, 1993; Kraemer & Adams, 2014; see supporting information Table S1). While mammals are known to use non-visual senses while foraging (e.g., smell: Pyare & Longland, 2001;

Hughes, Price & Banks, 2010; but see Discussion), they also utilize visual cues during the predation process. As such, it is of interest to determine whether mammalian predators differentially attack *P. cinereus* color morphs in accordance with several hypotheses in which predator behavior is mediated by visual cues (see *Expected Attacks For Each Hypothesis* below). We tested predictions in the field using clay replicas of salamander morphs that were exposed to mammalian predators for four to five days. At the conclusion of this period, we collected the replicas, scored them for mammalian attacks, and compared observed attack rates to attack rates expected under each hypothesis (described below).

*Replica Construction*

We constructed clay replicas out of Sculpey III® polymer clay. This clay does not harden under field conditions but retains impressions made by potential predators, thus serving as a record of attack events over several days. Similar approaches have been used to document predator-mediated selection on color patterns in diverse taxa, including snakes (Madsen, 1987; Pfennig, Harcombe & Pfennig, 2001), lizards (Husak *et al.,* 2006), mice (Vignieri, Larson & Hoekstra, 2010), frogs (Noonan & Comeault, 2009; Willink *et al*., 2014; McElroy, 2015), and salamanders (e.g. Brodie, 1993; Kuchta, 2005). We formed replicas to resemble *P. cinereus* morphs that were present at the four localities (striped, unstriped, and erythristic; Figure 2A-C). To construct the replicas, we hand-shaped 1.5 grams of black or red clay to resemble a basic salamander form with a head and tapered body (Figure 2D) . We made 300 replicas of each salamander color morph, for a total of 900 clay replicas.

*Replica Distribution and Retrieval*

We chose four locations in western Massachusetts based on the presence and frequency of *P. cinereus* color morphs, which we estimated by visiting each locality on three separate occasions in May and June of 2011. During each visit we searched under cover objects (e.g. logs, rocks, leaf litter) for one hour and noted each salamander encounter. In total, we observed 247 salamanders (72 at Fisk, 97 at Lily, 56 at Coys, and 22 at Palmer). At two locations striped and erythristic *P. cinereus* are present (Fisk and Lily; located in Fisk Meadows Wildlife Management Area and Lily Pond Wildlife Management Area, respectively), while at the other two locations striped and unstriped *P. cinereus* are frequently encountered (Coys and Palmer; located in Coys Hill Wildlife Management Area and Palmer Wildlife Management Area, respectively; Figure 3A). At three of the four localities *N. viridescens* are regularly encountered, while at the fourth locality (Coys) *N. viridescens* have been observed on the road directly adjacent to the wildlife management area. Historical records of morph frequencies in this region (Tilley *et al*., 1982) are similar to modern morph frequencies (A. Kraemer, *unpublished*), suggesting that morphs we identify as novel are likely to be the same as those local mammals would identify as novel. We placed the replicas in the field from 28 May to 31 May 2012, and we collected the replicas from 1 June to 5 June 2012 such that each replica was exposed to predators for four to five days. At each locality there was complete overhead canopy, while understory vegetation was sparse. Although there was some variation in understory coverage within sites, the large number of replicas distributed at each site ($N = 225$) led to even replica placement across all possible understory densities.

We distributed an equal proportion of each color morph at all four localities, so any deviation from equal predation across morphs strongly suggests that predators are using coloration to identify potential prey. We placed 15 replicas (five of each morph) along 75 m transects, each separated by 5 m. All replicas were placed directly on leaf litter five meters perpendicular to the transect line. Each locality had 15 transects and all transects had at least 20 m between them. After four (for Fisk Meadows) or five days (for Lily Pond, Coys Hill, and Palmer) replicas were retrieved, though not all replicas could be recovered (Fisk: 28 lost; Lily: 3; Coys: 6; Palmer: 8). This may be due in part to predators removing replicas from the transects, but may also be in part a result of our inability to relocate replicas placed in heterogeneous terrain (e.g. among boulders or vegetation). In such cases where models could not be recovered, they were removed from the analysis. We then scored replicas as either attacked if mammalian incisors or other indications of teeth could be identified in the clay, or not attacked if mammalian marks were absent (for similar scoring procedures, see: Brodie, 1993; Pfennig *et al.,* 2001; Kutcha, 2005; Low *et al.,* 2014). Raw totals are given in supporting information Table S2.

*Expected Attacks for Each Hypothesis*

Figure 1 provides a general schematic of attack frequencies under each predation hypothesis. To quantitatively characterize these into the expected attack rates under each hypothesis for our dataset, the following procedure was utilized.

*Mimicry Hypothesis* – The Mimicry Hypothesis states that predators will avoid attacking prey that resemble unpalatable models (Bates, 1862). Thus, the most parsimonious expectation for the Mimicry Hypothesis is predators will avoid mimics and attack the two non-mimetic morphs at equal frequency. Because no prior data was available for this taxon from which to generate prior expectations under this hypothesis, we consequently set expected attack rates at: 50% of observed attacks on unstriped replicas, 50% on striped replicas, and 0% on erythristic replicas (Figure 3B).

*Conspicuousness Hypothesis* – The Conspicuousness Hypothesis states that attacks will be distributed among prey such that the most conspicuous prey morphology will be attacked at the greatest frequency (Endler, 1978). We used previously published estimates of spectral reflectance for salamander morphs (collected from the mid-dorsal point of over 1000 salamanders; Kraemer & Adams, 2014), a local background (wet leaf litter; Kraemer & Adams, 2014), a measure of nocturnal irradiance (Veilleux & Cummings, 2012), and the achromatic spectral sensitivities of a mammal (Jacobs *et al.,* 1993) to estimate salamander conspicuousness for a nocturnal mammal (*sensu* Vorobyev *et al.,* 1998). This approach estimates the discriminability of visual signals from the signal to noise ratio of predator photoreceptors (Vorobyev *et al.,* 1998). The units are calculated as just-noticeable-difference units (jnd), where 1 jnd is the difference necessary for predators to detect a distinction between two spectra. Erythristic *P. cinereus* are most conspicuous (conspicuousness score = 3.59 jnd), unstriped *P. cinereus* are moderately conspicuous (conspicuousness score = 3.28 jnd), and striped *P. cinereus* are least conspicuous (conspicuousness score = 3.07 jnd; see Kraemer & Adams, 2014).

Although all color morphs were conspicuous against wet leaf litter, there were clear differences in conspicuousness among morphs. From these estimates of relative conspicuousness we set expected attack rates at: 36.1% on erythristic replicas, 33% of attacks on unstriped replicas, and 30.9% on striped replicas (Figure 3C).

Apostatic Selection *Hypothesis* – The Apostatic Selection Hypothesis states that most attacks will occur on morphs that are most common in the environment, such that common morphs will be attacked frequently, rare morphs will be attacked rarely, and novel morphs will be avoided (Allen, 1988). We thus set separate expected attack rates for each morph by locality (Figure 3D), with expected attack rates corresponding to the relative frequency of each morph naturally found at that locality (Figure 3D).

*Novel Avoidance Hypothesis* – The Novel Avoidance Hypothesis states that predators will avoid morphs not previously encountered (Marples *et al.,* 2007). We classified morphs naturally present at a locality as 'previously encountered' by predators and thus predicted that they would not be avoided. Conversely, we considered novel morphs at a locality as 'not previously encountered' and predicted that they would be avoided by predators. For example, at the Fisk locality unstriped *P. cinereus* are absent, while striped and erythristic salamanders are present. We thus set attack rates at Fisk as 0% of observed attacks on unstriped replicas, 50% attacks on striped replicas, and 50% attacks on erythristic replicas (Figure 3E).

*Non-Visual Hypothesis* – The Non-Visual Hypothesis states that color pattern will not influence attacks on potential prey (Endler, 1986). This hypothesis is derived from cases where predators locate prey items primarily using other senses (such as smell) or if visual cues not related to coloration are utilized. As such, the Non-Visual Hypothesis predicts equal attacks across color morphs, or 33% of the attacks on unstriped replicas, 33% on striped replicas, and 33% on erythristic replicas.

*Combined Hypothesis* – Finally, the Combined Hypothesis states that several of these factors jointly influence predator attacks on different color morphs, and thus predicts attack rates intermediate between the above predictions (Figure 3F; see *Combined Hypothesis* below). For example, if predators avoid mimics and unfamiliar color morphs, attacks will be distributed between non-mimetic and locally abundant color morphs.

**Statistical Analyses**

We evaluated observed predation rates on salamander replicas relative to alternative hypotheses using several analytical approaches. First, we calculated the binomial log-likelihood of each hypothesis given the observed data by setting $n$ as the number of retrieved replicas for a given morph, $x$ as the number of replicas that were attacked, and $p$ as the expected attack rate on that morph for a particular hypothesis (Sokal & Rohlf, 2012). Second, we determined which hypotheses provided the best 'fit' to the observed data using AIC scores. Next, we performed a series of pair-wise likelihood ratio tests (LRTs). These tests compared the likelihood of the observed data given each model of

predator behavior through a non-nested LRT approach (see *Simulation LRT*). Finally, because not all biological hypotheses were mutually exclusive, there existed the possibility that some combination of these hypotheses provided the best explanation of the observed data. We therefore developed a novel likelihood procedure to combine non-exclusive biological hypotheses (see *Combined Hypothesis* below).

*Simulation LRT*

Simulation LRT is an approach where non-nested models may be compared using likelihood ratio tests (Williams, 1970; Lewis, Butler & Gilbert, 2011). The procedure consists of several steps, which are outlined briefly here. First, for two non-nested models, 'A' and 'B,' calculate the likelihood of models 'A' and 'B' given the parameters of the models and observed data. Next, simulate a large number of datasets under the null model 'A,' fit each dataset to the null 'A' and alternative 'B' models, and calculate Likelihood Ratio Test Statistics (LRTS) for each simulated dataset as well as the observed dataset. The proportion of LRTS from the simulated datasets that are more extreme than the observed data is then estimated, and if the observed LRTS is more extreme than 95% of the simulated datasets, the LRT is judged as 'significant.' Finally, the steps are repeated with the role of 'null' and 'alternative' model reversed.

The procedure above provides a means of evaluating the fit of data to multiple models that are not statistically nested (as is the case here). As noted by Lewis *et al.* (2011), this analysis has four potential outcomes. 1) The LRT with A as the null model is non-significant, but the LRT with B as the null is significant. In this case, model A is a better

fit than model B. 2) The LRT with B as the null model is non-significant, but the LRT with A as the null is significant. In this case, model B is a better fit than model A. 3) If both LRTs are significant, neither model fits the data well. 4) If neither LRT is significant, the two models cannot be distinguished given the available data. We used this procedure on each pair of the hypotheses described above to determine which hypothesis (if any) provided a better explanation for the observed attack rates.

*Combined Hypothesis*

The LRT procedure described above is quite flexible, in that it allows one to compare the fit of non-nested models to data using likelihood ratio tests. However, the method assumes that all models are mutually exclusive, which is not always case. With respect to predation, predators may use prey color patterning differently throughout the predation process, which suggests the above hypotheses (Conspicuousness, Apostatic Selection, Mimicry, Novel Avoidance) are not necessarily exclusive of one another (Endler, 1986). Because of this, predators may, in essence, combine information from multiple sources, effectively utilizing multiple strategies that fall within the previously defined hypotheses. In such cases, the best explanation for the observed attack rates would be from a model that does not exclusively describe one or another scenario, but rather combines multiple models (akin to model averaging using AIC weights). To address this possibility, we developed a likelihood procedure that, given a set of explanatory models, identified the best fitting model (based on likelihood) where this model consisted of combinations of the previously stated hypotheses. Procedurally, this was accomplished by incorporating weights (w) for each model, which were multiplied by the parameters of each hypothesis

(i.e. Conspicuousness: $\beta_{conspic}$, Apostatic Selection: $\beta_{apo}$ , Mimicry: $\beta_{mim}$, Novel Avoidance: $\beta_{novel}$). These weights were then adjusted to maximize the likelihood of a combined hypothesis, with the constraint that the weights sum to 1.0. Thus, the best fitting combined model was found as:

$$\beta_{combined} = w_{conspic}\beta_{conspic} + w_{freq}\beta_{apo} + w_{mim}\beta_{mim} + w_{novel}\beta_{novel},$$

with $\beta_{combined}$ signifying the parameters of a combined model that maximizes the likelihood of a hypothesis incorporating aspects of each single hypothesis. Note that for a 'pure' model, the weight for that component would be w = 1.0, and the weights for the remaining model contributions would be w = 0.0. We implemented this procedure using the 'optim' function in the 'stats' package found in 'R.' We then compared the fit of the combined hypothesis to each 'pure' hypothesis using likelihood, Akaike Information Criterion (AIC), and simulation LRT. All analyses were conducted in R 3.2.2 (R Development Core Team, 2015). Associated R code for this procedure can be found in supporting information S3.

## Results

*Likelihood and AIC*

We found likelihood scores and AIC scores reflected similar patterns of fit among single models (Table 1). In both cases, the Novel Avoidance Hypothesis was a much better fit to the observed data than were the remaining models. The next best-fitting models (Non-

Visual and Conspicuousness) were >6 ΔAIC units from the Novel Avoidance Hypothesis, implying that the Novel Avoidance Hypothesis represented a substantially better fit to the observed attack rates (Table 1). Further, two models that described strict avoidance of one morph (Mimicry and Apostatic Selection hypotheses) provided the poorest fit to the data, implying that these models did not describe how predators attacked potential prey in this system (Table 1).

*Simulation LRT*

Pairwise comparisons of models through simulation LRT corroborated the findings described above, indicating that the Mimicry and Apostatic Selection hypotheses were particularly poor predictors of the observed data (Table 2). In no comparisons did either of these models fit better than the alternative. Comparisons between the remaining single models were more equivocal, and we were unable to determine whether the Novel Avoidance, Conspicuousness, or Non-Visual models provided the best fit.

*Combined Hypothesis*

Interestingly, the combined hypothesis that best predicted our observed data was most strongly influenced by the Conspicuousness Hypothesis ($w_{conspic} = 0.61$) and Novel Avoidance Hypothesis ($w_{novel} = 0.39$), with no contribution from the remaining hypotheses. All other combinations of hypotheses, including those that included the Non-Visual Hypothesis, provided a poorer fit to the data, regardless of their AIC scores as calculated singly. Consequently, only the parameters from the Conspicuousness and Novel Avoidance hypotheses were considered in calculating the AIC score for the

combined hypothesis. Note that the best-fitting combined hypothesis does not weight the Conspicuousness and Novel Avoidance hypotheses according to AIC or likelihood scores, but by the predictions made by each resulting model. Both likelihood and AIC scores indicated that the Combined Hypothesis was a far better predictor of our observed data than any single hypothesis (Table 1). Likewise, the Combined Hypothesis was a better fit than any single hypothesis as indicated by simulation LRT (Table 2).

Overall, results from AIC, simulation LRT, and combined LRT consistently group the Novel Avoidance Hypothesis among the best-fitting hypotheses. Results from the combined LRT test found the highest support for the combination of the Novel Avoidance and Conspicuousness hypotheses. It should be noted here that although our clay replicas appeared similar to the salamander color morphs they represented, there was a possibility that dichromatic mammals viewed them differently. To account for this we recalculated all analyses using conspicuousness estimates of clay reflectance in the place of salamander reflectance (in this case, striped morph conspicuousness was estimated as the average reflectance of red and black clay: results not shown). Under this formulation the Conspicuousness Hypothesis yielded a somewhat poorer fit to the observed data and was excluded from the combined model.

## Discussion

Selection on color pattern can be strongly driven by predators (Endler, 1988), yet how multiple predators influence selection on the same color patterns is understudied (Endler,

1986). Importantly, prey color patterns that signify toxicity to some predator species may not carry the same indication of unpalatability to other predators (Pekár *et al.,* 2011; Willink *et al.,* 2014). In this study, we tested several hypotheses of selection on color pattern between mammal predators and the salamander *Plethodon cinereus*. We found consistent support for the single hypothesis that mammals avoid novel and unfamiliar prey, an observation that has been made in experimental settings (Chitty & Kempson, 1949; Mitchell, 1976), and we found the highest support for a combined hypothesis in which mammalian predators preferentially attack the most conspicuous prey while avoiding unfamiliar color morphs.

Avian predators avoid erythristic *P. cinereus* because they appear similar in coloration to the toxic salamander *Notophthalmus viridescens* (Brodie & Brodie, 1980; Tilley *et al.,* 1982). Selection for Batesian mimicry in *P. cinereus* may or may not extend to additional predator species, such as mammals. We tested this hypothesis by fitting our observed data to a model in which mammals avoided erythristic replicas while not discriminating between either striped or unstriped replicas. We found no support for this hypothesis alone or as a component of the Combined Hypothesis. The lack of support for mimicry between erythristic *P. cinereus* and *N. viridescens* from the perspective of mammalian predators suggests that mammals do not associate erythristic *P. cinereus* with unpalatability, which aligns well with recent research in the system. For example, Kraemer and Adams (2014) found evidence that the dichromatic mammalian visual system allows mammals to use visual stimuli to differentiate salamander species from each other and common backgrounds on the basis of brightness, but not coloration. This

is significant with regards to mimicry because erythristic *P. cinereus* and *N. viridescens* are similar with respect to color, while they differ substantially with respect to brightness (Kraemer & Adams, 2014). Predators that select for mimicry between *P. cinereus* and *N. viridescens* must be capable of identifying prey by color while foraging, which monochromatic and dichromatic mammals appear unable to do. When paired with our findings, this research strongly indicates that monochromatic and dichromatic mammals do not select for mimicry in this system.

By contrast, in this study we found strong statistical support for a composite hypothesis that incorporates aspects of two modes of prey selection that utilize visual information, Novel Avoidance (Marples *et al.,* 2007) and Conspicuousness (Endler, 1978), with Novel Avoidance previously observed in mammal taxa (Chitty & Kempson, 1949). Mammals are well known to use olfactory cues while foraging (Pyare & Longland, 2001; Hughes *et al.,* 2010). However, our data support two hypotheses that each indicate mammals also utilize visual cues, specifically by attacking prey that most strongly contrast with local backgrounds and that are most familiar in appearance. This result implies that mammals use visual cues both during the detection and identification phase of foraging, which allows for the possibility that these predators use visual cues of their prey at different stages of a single predation event (Endler, 1986). For example, a color pattern that influences a predator's ability to first detect potential prey (the Conspicuousness Hypothesis; Endler, 1978), may also impact whether that predator then recognizes the potential prey as a prey item (i.e. the Novel Avoidance, Batesian Mimicry, or Apostatic Selection hypotheses; summarized in Endler, 1986). Furthermore, both our approaches to

estimate salamander conspicuousness suggest that, although conspicuousness may be important, novel avoidance likely plays a far more important role in determining attack likelihood on salamanders by mammal predators. Our findings indicate selection on *P. cinereus* by mammalian predators is complex, with a combination of directional selection favoring inconspicuous individuals and frequency-dependent selection favoring novel color morphs. Interestingly, this selection could result from a complex predator community consisting of several species with each species responding to salamander visual cues differently, a predator community with behavioral plasticity among individuals, or a single predator type that incorporates visual cues relating to prey conspicuousness and novelty at every encounter with potential prey. The next, and perhaps more technically challenging, research direction will be to identify all members of these salamander predator communities and characterize how each interacts with individual salamander prey.

Salamander conspicuousness and novelty to predators are strongly influenced by local community structure and environmental factors, which may result in selection pressures that differ among localities. For example, conspicuousness is determined by the contrast between prey and background (Endler, 1978). Importantly, background may differ by locality and therefore be influenced by factors such as the composition of tree species, precipitation, vegetative cover, and time of year (Endler, 1993). Likewise, the contribution of Novel Avoidance in the best-fitting combined hypothesis supports the hypothesis that predators avoid prey they have not previously encountered (e.g. Mitchell, 1976; Lindström *et al.,* 2001). Furthermore, when classified as either local (if

corresponding *P. cinereus* morphs were present at the locality) or novel (if corresponding *P. cinereus* morphs were absent), local salamander replicas were twice as likely to be attacked than novel replicas (local proportion attacked = 0.23; novel proportion attacked = 0.11). Thus, our Combined Hypothesis indicates that mammal predators are strongly influenced by local factors. Variation in potential backgrounds and prior predator experience may contribute to the variability observed between natural predator-prey studies conducted under similar, but not identical conditions (e.g., as seen between Saporito *et al.,* 2007 and Hegna, Saporito & Donnelly, 2013).

*Plethodon cinereus* are likely hunted by several predator taxa (Lotter & Scott, 1977), with each species potentially directing the evolution of *P. cinereus* phenotype along a different evolutionary trajectory. Previous research suggests that bird predators select *P. cinereus* coloration such that erythristic individuals evolve coloration that is similar to *N. viridescens* (Brodie & Brodie, 1980; Tilley *et al.,* 1982; Kraemer & Adams, 2014). Here, we present evidence that mammal predators may select for inconspicuousness in *P. cinereus* as well as for novel color morphs. This complex selective regime may contribute to the maintenance of polymorphism in *P. cinereus* through variation in predator communities within and among localities as well as consistent selection for novel, unfamiliar color morphs. Research on other taxa has likewise found support for frequency-dependent selection (Pfennig *et al.,* 2007; Karpestam, Merilaita & Forsman, 2014), though under different mechanisms (i.e. apostatic selection and limited attention, respectively). Selection on prey that depends on the relative abundance of different morphologies is relatively understudied, though findings like these suggest that

mechanisms such as these may play a large role in maintaining phenotypic diversity.

Color pattern polymorphism is widespread in many species in addition to *P. cinereus* (e.g. frogs: Hoffman & Blouin, 2000; moths: Poulton, 1890; land snails: Cain & Sheppard, 1954; reptiles: Norris & Lowe, 1964, Olsson, Stuart-Fox & C Ballen, 2013; insects: Karpestam *et al.,* 2014), though the mechanisms maintaining polymorphisms are hotly debated (Bond, 2007). Polymorphisms can be maintained by frequency-dependent selection (such as novel avoidance or limited attention; Marples *et al*., 2007; Karpestam *et al.,* 2014), variation in prey community structure (Merilaita & Kaitala, 2002), multiple predators with variable behavior (Nokelainen *et al.,* 2014), gene flow among populations (Slatkin, 1987), or habitat heterogeneity (Sandoval & Nosil, 2005). In many species, including *P. cinereus*, polymorphism is likely maintained by a complex bricolage of the above mechanisms. The results of this study demonstrate that prey selection by predators is complex and is best understood when considering the joint influence of multiple non-exclusive hypotheses.

Analyses that combine hypotheses as we have done have important strengths over traditional analyses that compare single hypotheses. First, many biological phenomena are subject to multiple factors that interact in complex ways (e.g. Prum & Brush, 2002). Hypotheses that can account for such interactions have the potential to make biological predictions that more closely approximate biological reality. Second, analytical frameworks like our combined analysis can be used to propose hypotheses regarding the relative importance of different, non-exclusive phenomena. For example, our combined

analysis found that the familiarity and conspicuousness of prey was more important for predicting mammal attack rates than the similarity of prey to other, toxic species. These hypotheses can then be further tested in more explicit studies. Similar combined approaches can be used to disentangle other, complex phenomena such as community assembly, colonization processes, and adaptive landscapes.

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Potential Conflicts of Interest:

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**Table 1**. Likelihood and AIC scores for each hypothesis. Both approaches indicate that, of the hypotheses tested, Novel Avoidance is the best fitting single hypothesis, while the Combined Hypothesis best fits the observed data over all single hypotheses.

| **Model** | **-lnL** | **AIC** |
|---|---|---|
| Combined | -3.76 | 15.52 |
| Novel Avoidance | -7.65 | 19.31 |
| Non-Visual | -10.58 | 25.16 |
| Conspicuousness | -10.93 | 25.86 |
| Mimicry | -19.18 | 42.36 |
| Apostatic Selection | -35.14 | 74.28 |

**Table 2**. Pairwise comparisons of each hypothesis using simulation-based LRT. 'Apostatic' refers to the Apostatic Selection Hypothesis, 'Conspic.' refers to the Conspicuousness Hypothesis, 'Novel Avoid.' refers to the Novel Avoidance Hypothesis, and 'Non-V' refers to the Non-Visual Hypothesis. Row names that correspond to each cell indicate the model treated as the 'null' hypothesis, while column names denote the 'alternative' hypothesis. Note that the Combined Hypothesis is the best fit among all comparisons, because all comparisons with the Combined Hypothesis as the null are non-significant and all comparisons with the Combined Hypothesis as the alternative are significant.

| | Apostatic | Mimicry | Conspic. | Novel Avoid. | Non-V | Combined |
|---|---|---|---|---|---|---|
| Apostatic | - | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| Mimicry | **<0.001** | - | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| Conspic. | 0.120 | 0.540 | - | **0.005** | 0.420 | **<0.001** |
| Novel Avoid. | 0.798 | **<0.001** | **<0.001** | - | **<0.001** | **<0.001** |
| Non-V | 0.343 | 0.877 | 0.991 | **0.020** | - | **<0.001** |
| Combined | 0.775 | 0.552 | 0.922 | 0.616 | 0.766 | - |

**Figure 1.** Schematic of predator attacks expected under each single hypothesis. A) The Mimicry Hypothesis states that mimics will be avoided, resulting in 100% of attacks on non-mimetic morphs. B) The Conspicuousness Hypothesis states that prey with the highest contrast with background will be attacked most. In this example, the gray morph is twice as conspicuous as the black morph, thus sustaining twice as many attacks. C) The Apostatic Selection Hypothesis states that rare morphs will be attacked less frequently and common morphs will be attacked more. In this example, the gray morph is rare (making up 25% of the local population and thus 25% of total attacks), while the black morph is more common (making up 75% of the local population and thus 75% of total attacks). D) The Novel Avoidance Hypothesis states that morphs not locally encountered are avoided. In this example, the gray morph is locally encountered by predators and thus attacked, while the black morph is absent and is thus avoided by predators. Note that the Non-Visual Hypothesis predicts no difference in attacks among morphs (not depicted), and the Combined Hypothesis has different predictions depending on the weights given for each single hypothesis (not depicted).

**Figure 2.** *Plethodon cinereus* color morphs, clay replicas, and typical mammalian impressions. A) An erythristic *P. cinereus* salamander; B) a striped *P. cinereus* salamander; C) an unstriped *P. cinereus* salamander. D) Representative clay replicas of each salamander morph: Left, erythristic; center, striped; right, unstriped. Typical mammalian impressions on an E) unstriped replica and an F) striped replica.

**Figure 3.** Predator attacks A) observed, B) predicted under the Mimicry Hypothesis, C) the Conspicuousness Hypothesis, D) the Apostatic Selection Hypothesis, E) the Novel Avoidance Hypothesis, and F) the Combined Hypothesis. Black bars represent the unstriped color morph, striped bars represent the striped morph, and red bars represent the erythristic morph. The pie charts in panel 'A' denote the proportion of each *Plethodon cinereus* color morph locally present at each locality. Fisk: 28% erythristic and 72% striped; Lily: 21% erythristic and 79% striped; Coys: 38% unstriped and 62% striped; Palmer: 18% unstriped and 82% striped.

## Appendix S1: Potential Mammalian Predator Visual Abilities

**Table S1.** Potential mammalian predators of *Plethodon cinereus* listed by family, including their activity patterns and known visual systems. List taken from http://www.mass.gov/eea/agencies/dfg/dfw/fish-wildlife-plants/state-mammal-list.html Sources are listed below table.

| Family | Time Active | Visual System |
|---|---|---|
| Canidae (foxes and coyotes) | mostly nocturnal | dichromatic |
| Cricetidae (deermice and voles) | varies | unknown |
| Didelphidae (oppossums) | nocturnal | monochromatic |
| Dipodidae (jumping mice) | nocturnal | unknown |
| Erethizontidae (porcupines) | nocturnal | unknown |
| Felidae (cats) | varies | dichromatic |
| Mephitidae (skunks) | nocturnal | unknown |
| Muridae (old world rats and mice) | varies | dichromatic |
| Mustelidae (weasels) | varies | dichromatic |
| Procyonidae (racoons) | nocturnal | monochromatic |
| Sciuridae (squirrels) | diurnal (squirrels)<br>nocturnal (flying squirrels) | dichromatic<br>monochromatic |
| Soricidae (shrews) | nocturnal | dichromatic |
| Talpidae (moles) | varies | dichromatic |
| Ursidae (bears) | varies | dichromatic |

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## Appendix S2: Clay Replicas Recovered and Attacked

**Table S2. Number of replicas attacked and recovered at each locality. Values above the slash indicate the number attacked, while values below the slash indicate the total number recovered (from a possible 75).**

| Locality | Unstriped | Striped | Erythristic |
|---|---|---|---|
| Fisk | 9/61 | 14/66 | 17/70 |
| Lily | 7/73 | 12/74 | 20/75 |
| Coys | 11/73 | 7/73 | 3/73 |
| Palmer | 10/72 | 18/72 | 9/73 |

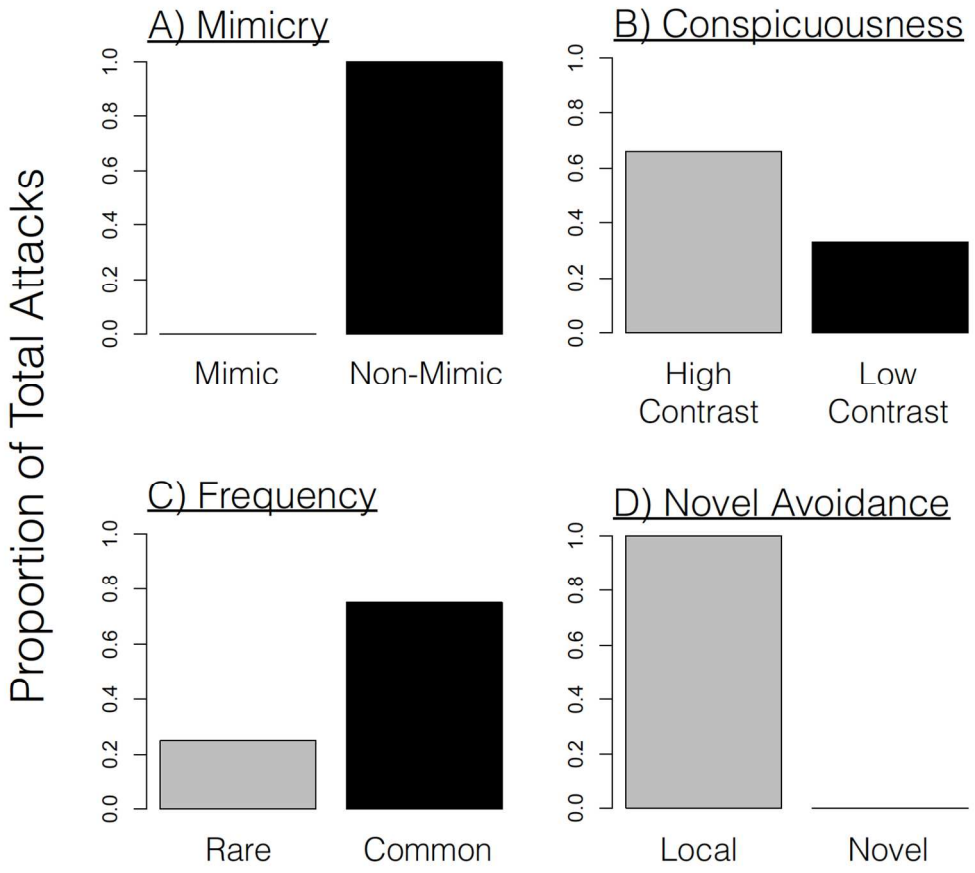
## Appendix S3: R Code For Combined Analysis

Note: this code is organized for the data presented in this paper, but can be reformulated for any other data/hypothesis structure for which like likelihood can be calculated.

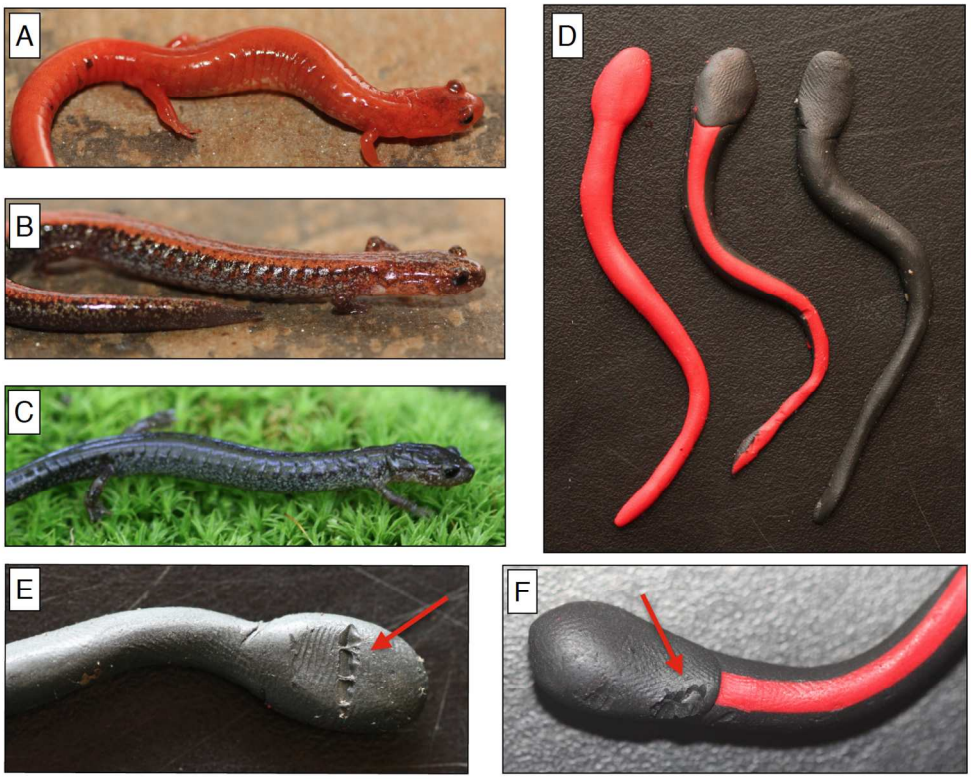
```
# General order of operations:
    # 1) Set up data
    # 2) Combine models
      # 3) Find optimum combination of models to maximize likelihood
      # 4) Identify weights
      # 5) Calculate new predictions

#################### Step 1 ####################
obs.x<- #vector of 'successes' in n trials
obs.y<- #vector of 'failures in n trials
n<- #number of trials
A.x.exp<- #expected number of 'successes' under hypothesis 'A'
B.x.exp<- #expected number of 'successes' under hypothesis 'B'
x.exp<-cbind(A.x.exp, B.x.exp)
p.exp<- #similar matrix to x.exp, but with expected proportion of 'successes' under each hypothesis
p.obs<- #observed proportion of 'successes'

#################### Step 2 ####################
```

```
propAB<-function(a,b){
    tot<-sum(a,b)
    a<-a/tot; b<-b/tot
    return(c(a,b))
}
comb.like.fit<-function(AB){
    abVec<-propAB(AB[1],AB[2])
    p.combine<-(abVec[1]*P.exp[,1] + abVec[2]* P.exp[,2])
    comb.like<-log((p.combine/p.obs)^obs.x*((1-p.combine)/(1-p.obs))^(obs.y))
    mask<-apply(as.matrix(comb.like),2,is.infinite) #finds all 'Inf'
    comb.like2<-comb.like
    comb.like2[mask]<-0 #replaces 'Inf' with 0 (because integer/0 can not be
      calculated; this should       result in a more conservative likelihood for all model-
      dataset combinations)
    mask<-apply(as.matrix(comb.like2),2,is.nan) #finds all 'NaN'
    comb.like3<-comb.like2
    comb.like3[mask]<-0 #replaces 'NaN' with 0 (these were all instances in which 0/0
      = NaN)
    result<-sum(comb.like3)
    return(result)
}

#################### Step 3 ####################
```

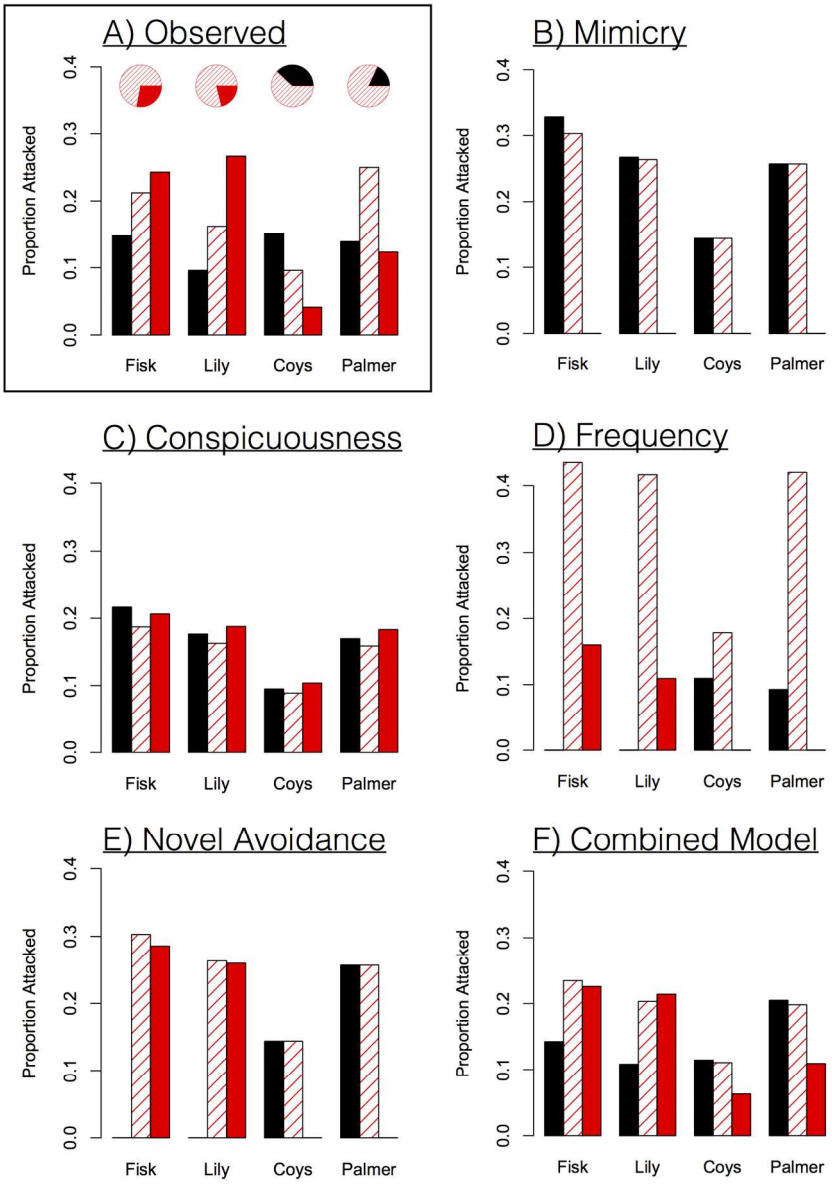
```
AB<-c(1,1)
comb.like.fit(AB)
optim(AB,comb.like.fit,method="L-BFGS-B",control=list(fnscale=-1),lower=0)
solution<-optim(AB,comb.like.fit,method="L-BFGS-B",control=list(fnscale=-
        1),lower=0)$par
solution

#################### Step 4 ####################
A.weight<-solution[1]/sum(solution)
B.weight<-solution[2]/sum(solution)

#################### Step 5 ####################
combined.p.exp<-A.weight*p.exp[,1]+B.weight*p.exp[,2]
```




Schematic of predator attacks expected under each single hypothesis. A) The Mimicry Hypothesis states that mimics will be avoided, resulting in 100% of attacks on non-mimetic morphs. B) The Conspicuousness Hypothesis states that prey with the highest contrast with background will be attacked most. In this example, the gray morph is twice as conspicuous as the black morph, thus sustaining twice as many attacks. C) The Apostatic Selection Hypothesis states that rare morphs will be attacked less frequently and common morphs will be attacked more. In this example, the gray morph is rare (making up 25% of the local population and thus 25% of total attacks), while the black morph is more common (making up 75% of the local population and thus 75% of total attacks). D) The Novel Avoidance Hypothesis states that morphs not locally encountered are avoided. In this example, the gray morph is locally encountered by predators and thus attacked, while the black morph is absent and is thus avoided by predators. Note that the Non-Visual Hypothesis predicts no difference in attacks among morphs (not depicted), and the Combined Hypothesis has different predictions depending on the weights given for each single hypothesis (not depicted).
274x245mm (144 x 144 DPI)




*Plethodon cinereus* color morphs, clay replicas, and typical mammalian impressions. A) An erythristic *P. cinereus* salamander; B) a striped *P. cinereus* salamander; C) an unstriped *P. cinereus* salamander. D) Representative clay replicas of each salamander morph: Left, erythristic; center, striped; right, unstriped. Typical mammalian impressions on an E) unstriped replica and an F) striped replica.
279x220mm (144 x 144 DPI)




Predator attacks A) observed, B) predicted under the Mimicry Hypothesis, C) the Conspicuousness Hypothesis, D) the Apostatic Selection Hypothesis, E) the Novel Avoidance Hypothesis, and F) the Combined Hypothesis. Black bars represent the unstriped color morph, striped bars represent the striped morph, and red bars represent the erythristic morph. The pie charts in panel 'A' denote the proportion of each *Plethodon cinereus* color morph locally present at each locality. Fisk: 28% erythristic and 72% striped; Lily: 21% erythristic and 79% striped; Coys: 38% unstriped and 62% striped; Palmer: 18% unstriped and 82% striped.
300x422mm (150 x 150 DPI)